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<b>(54) Title:</b> PROCESS FOR PRODUCTION OF HUMAN MILK FAT REPLACERS  <b>(57) Abstract</b>  Triglycerides with more than 40 wt% saturated fatty acids in the 2-position contain considerable amounts of trisaturated triglycerides; these trisaturated triglycerides are removed (reduced) by performing an enzymic conversion with a source providing unsaturated C <sub>18+</sub> residues, using a 1,3-specific enzyme.		

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## PROCESS FOR PRODUCTION OF HUMAN MILK FAT REPLACERS

The enzymic preparation of fats that can be used as human milk fat replacers, in which fats more than 40 wt.% of the total amount of saturated fatty acids present are in the 2-position, is the subject of our earlier European patent 0209327 (Application N° 86305325.2) and European patent application 91300496.6.

According to these processes, fats (A) high in trisaturated triglycerides ( $= S_3$ , wherein S is preferably palmitic) are converted with a source (B) that provides oleic acid moieties. Sources of B are, e.g., free fatty acid mixtures rich in oleic acid or triglycerides with a high oleic acid content in the 1,3-positions, e.g. high-oleic sunflower oil.

The conversion is carried out in the presence of a 1,3-specific enzyme. The product of this enzymic conversion containing residual amounts of non-converted  $S_3$ , partial conversion products, such as SSO, and the desired conversion products (OSO), is subjected to a fractionation process in which a product rich in OSO is obtained while a product rich in SSO is removed and recirculated to the conversion zone. Spent oleic acid sources (B) are removed in a strip zone and can be used again in the process, if and when appropriate.

Human milk replacement fats can only contain very limited amounts of trisaturated triglycerides ( $S_3$ , where S = saturated fatty acid with at least 16 C-atoms). When the amount of  $S_3$  is too high, the fat becomes too hard, and simultaneously absorption of the fat by infants is affected adversely.

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However, the products obtained in the enzymic conversion

zone normally still contain amounts of 7 or more wt.% of  $S_3$ , which is above the level, generally regarded as acceptable (about 4 wt%). Only when these products were subjected to solvent fractionation could these levels be  
5 decreased to the desired level. However, wet fractionation requires high investments in equipment, time and energy and is therefore less attractive from a commercial point of view.

10 We have now found a new process by which the desired fats of maximum levels of 3 wt.% of  $S_3$  are obtained and in which fractionation can be avoided.

Accordingly, our invention is concerned with a process for  
15 the preparation of triglyceride compositions, in which more than 40 wt.% of the total amount of saturated fatty acids present are in the 2-position, by enzymic interesterification of triglycerides high in trisaturates (= A) with a source (B) providing unsaturated fatty acid  
20 moieties ( $C_{18}$  or more), which process is characterized by the performance of an enzymic removal, using a 1,3-specific enzyme, of trisaturated triglycerides (=  $S_3$ ,  $S = C_{16}$  or higher), in particular trisaturated triglycerides high in  $P_3$  and/or  $St_3$  ( $P$  = palmitic,  $St$  = stearic) or a combination  
25 thereof (PSt P. etc.) from a product high in triglycerides rich in 2-saturated fatty acids from the USU and/or SSU type ( $U$  = unsaturated fatty acids  $C_{18}$  or more;  $S$  = saturated fatty acids  $C_{16}$  or more) by contacting the product rich in USU and/or SSU with an oil blend high in  
30 triglycerides with acids other than palmitic and/or stearic acid in the 1,3-positions, but not being a triglyceride composition with more than 40 wt% of the fatty acids in the 2-position being saturated fatty acids with 16 or more C-atoms.

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Preferably, blends are used which are rich in triglycerides having a high level of unsaturated fatty acids, such as

oleic or linoleic acid or short chain saturated fatty acids, such as  $C_{8:0}$ ;  $C_{10:0}$  or  $C_{12:0}$  in at least the 1,3-positions.

5 A preferred process is a multi-step process comprising the steps of :

- 1) converting triglycerides A enzymatically with a 1,3-specific enzyme and the unsaturated acid source B in a  
10 first enzymic conversion zone;
- 2) removing the spent unsaturated acid source B from the crude product of 1);
- 3) optionally subjecting the remaining part of 2) to an enzymic removal of diglycerides;
- 15 4) converting the remaining part of 2) and/or the product of 3) in a second enzymic conversion zone with a fresh source providing unsaturated acid moieties (B) in the presence of a 1,3-specific enzyme;
- 5) removing the spent unsaturated acid source B from  
20 the crude product of 4);
- 6) optionally recirculating the spent unsaturated acid source (B) from 5) to step 1);
- 7) decreasing the level of trisaturates ( $S_3$ ,  $S = S_{16}$  or higher) in the remaining part of 5) by a further enzymic  
25 treatment, using a 1,3-specific enzyme with an oil blend high in triglycerides with acids other than palmitic and/or stearic acid in the 1,3-positions, but not being a triglyceride composition with more than 40 wt% of the fatty acids in the 2-position being saturated fatty acids with 16  
30 or more C-atoms.

It is surprising to find in this case that a third enzymic conversion can replace the fractionation procedure, as the levels of  $S_3$  after two previous enzymic conversions were  
35 still too high. In an alternative embodiment of the process the second enzyme conversion (steps 4 and 5 above) can be omitted, proceeding directly to step 7 by employing a

sufficiently high ratio of acid to oil in step (1).

The above-mentioned process is in particular applicable to systems in which a fatty acid mixture high in oleic acid is  
5 used as source (B) providing oleic acid moieties.

Fats A, which can be used as fats high in trisaturates  $S_3$  (S= palmitic and/or stearic), are in particular the top fractions of palm oil fractionation. These fats preferably  
10 contain more than 60 wt.% of  $S_3$  (S = palmitic and/or stearic), while more than 20 wt.% of SSU (U = unsaturated) can also be present.

The best results are obtained when weight ratios of  
15 trisaturated fat A : unsaturated acid source B of 1:2 - 2:1 are applied in the first and/or the second enzymic conversion zones of steps 1) and/or 4).

The other process conditions in these enzymic zones can be  
20 chosen within the process conditions as disclosed in, e.g., GB 1,577,933, European patent 0209327 (86305325.2) and European patent application 91300496.6. In particular, water contents, water activity, solvent, selection of 1,3-specific enzyme, catalyst-supporting materials are  
25 mentioned in these documents.

As any enzymic conversion inevitably also leads to the formation of some diglyceride, it is very useful to subject the crude triglyceride products of the enzymic  
30 conversion(s) to a treatment with a catalyst specific for the conversion of diglycerides into glycerol. Very useful is an Amano G-catalyst, which is conventionally used for this purpose.

35 In step 7), the level of  $S_3$  is decreased by enzymic conversion, using the oil blend which is high in triglycerides with acids other than palmitic and/or stearic

acid in the 1,3-positions. It is very suitable to use for this purpose: medium chain triglycerides (i.e. MCT-oils, based on C<sub>8</sub>-C<sub>14</sub> fatty acids), coconut oil, palm kernel, soybean oil, palm oil, rapeseed oil, high-oleic sunflower  
5 oil, olive oil, fish oil, fungal, algal or other lipid sources rich in long chain polyunsaturated fatty acids, such as C<sub>20:4</sub> w 6 or C<sub>22:6</sub> w 3, and butterfat, or mixtures thereof.

10 As well as being suitable for applications in infant formulas and infant foods as human milk fat replacers, fats derived from the above process are readily digestible and may also be applied in other foods, for example in confectionery, spreads, creams, bakery products, cooking  
15 oils and health foods, and as a component in clinical products.

Our invention will be further explained by the following non-limiting Example(s).

EXAMPLE I

Palm stearine was reacted with high oleic sunflower acids (1:1 by weight) by passing the mixture through a column packed with SP-392. The product of this reaction was distilled to remove fatty acids and treated with Lipase G to reduce the diglyceride level. The residual  $S_3$  level in this product was 7.6%. This product was mixed with high oleic sunflower oil (1:1 by weight) and interesterified using SP-392 as catalyst. The silver phase HPLC analysis of the fat blend before and after interesterification is shown below:

wt%	SSS	SOS	SSO	SLnS	SOO	OSO	OSln	OOO	> 3DB
Physical Blend	3.8	1.6	12.9	-	12.6	11.6	8.4	41	5.8
After int <sup>n</sup>	2.4	4.2	12.7	0.7	20.9	15.2	4.9	3.9	33.7

S=  $\geq C_{16:0}$ , O= oleic, ln= linoleic, > 3DB= > 3 double bonds/triglyceride

The fatty acid composition of this blend (unaffected by interesterification) was:

Fatty

acids	12:0	14:0	16:0	18:0	18:1	18:2	18:3
wt%	0.2	0.6	22.6	3.8	63.5	7.7	0.1

Intesterification of the physical blend reduced the  $S_3$  level by nearly 40%.

EXAMPLE II

Palm stearine was mixed with fatty acids (normal and high oleic sunflower plus canola acids) in a weight ratio of 1:0.75, the feed partially saturated with water and passed through a column packed with immobilised lipase (mucor miehei on duolite) (Novo; code SP-392). The product of Step 1 was collected and the fatty acids removed by distillation (Step 2). Treatment of the oil fraction with

lipase G (diglyceride-specific lipase; Amano Pharmaceutical Co) was used to reduce diglyceride levels (Step 3). Fresh acids were added to the resultant triglycerides in the same ratio as before, and passed through a second enzyme column (Step 4). The fatty acids stripping and lipase G steps were repeated (steps 5 and 6). The resultant triglyceride (50 parts) was mixed with liquid vegetable oils (30 parts) and coconut oil (20 parts) and passed through a third enzyme column containing SP-392 catalyst step 7). The final oil blend was refined. Step 7 reduced the SSS level from 10% to 2.7% in the refined oil.

## Results

### 1. Product of Steps 4-6

#### 1.1 Fatty acid composition

	C <sub>16:0</sub>	C <sub>18:0</sub>	C <sub>18:1</sub>	C <sub>18:2</sub>	C <sub>18:3</sub>
Total	48.3	2.4	35	13.3	1.0
2-position	91.9	0.4	6	1.4	0.4

#### 1.2 Silver Phase HPLC

SSS	SSU	USU	SUS	SUU	UUU
10	40.4	42.3	0.8	3.2	3.4

### 2. Product of Step 7

#### 1.1 Fatty Acid composition

	C <sub>8:0-14:0</sub>	C <sub>16:0</sub>	C <sub>18:0</sub>	C <sub>18:1</sub>	C <sub>18:2</sub>	C <sub>18:3</sub>
Total	18	25	3.0	35.4	16.0	2.0
2-position	15.7	42.8	0.2	16.5	16.0	2.8

(57% of total palmitate in 2-position)

S<sub>3</sub> level reduced to 2.7% (S = C<sub>16:0</sub> + C<sub>18:0</sub>)

EXAMPLE III

Palm stearine (1 part) was mixed with unsaturated fatty acids (2 parts) derived from vegetable sources, partially wetted and reacted by passing through a column packed with SP-392 lipase (step 1). The product of this reaction was distilled to remove fatty acids (step 2) and treated with lipase G to reduce the diglyceride level (step 3). This product (50 parts) was mixed with 20 parts coconut oil and 30 parts mixed vegetable oils (sunflower, high oleic sunflower, canola, soybean) and reacted by passing through a second enzyme column (step 4). The final product was collected and fully refined. The  $S_3$  level after step 3 was 11.8%, and was reduced to 2.5% after step 4.

1. Product of step 3

## 1.1 Fatty acid composition

	C <sub>16:0</sub>	C <sub>18:0</sub>	C <sub>18:1</sub>	C <sub>18:2</sub>	C <sub>18:3</sub>
Total	51.1	2.3	35	10.5	1.1
2-position	95.4	0.2	4.0	0.4	0.4

## 1.2 Silver Phase HPLC

SSS	SSU	USU	SUS	SUU	UUU
11.8	43.8	40.4	0.4	1.8	1.9

## 2.1 Fatty Acid analysis

	C <sub>8:0-14:0</sub>	C <sub>16:0</sub>	C <sub>18:0</sub>	C <sub>18:1</sub>	C <sub>18:2</sub>	C <sub>18:3</sub>
FAME	18	25	3.0	35.2	17.0	1.6
2-position	18.7	43	0.2	17.3	19.0	1.8

(57% of total palmitate in 2-position)

$S_3$  level reduced to 2.4% ( $S = C_{16:0} + C_{18:0}$ )

CLAIMS

1. A process for the preparation of triglyceride compositions, in which more than 40 wt.% of the total amount of saturated fatty acids present are in the 2-position, by enzymic interesterification of triglycerides high in trisaturates (= A) with a source providing unsaturated fatty acid moieties with 18 or more C-atoms (= B), characterized by the performance of an enzymic removal, using a 1,3-specific enzyme, of trisaturated triglycerides (= S<sub>3</sub>, S= 16 or more C-atoms) from a product high in triglycerides, rich in 2-saturated fatty acids from the USU and/or SSU type by contacting the product rich in USU and/or SSU with an oil blend high in triglycerides with acids other than palmitic and/or stearic acid in the 1,3-positions, but not being a triglyceride composition with more than 40 wt% of the fatty acids in the 2-position being saturated fatty acids with 16 or more C-atoms

2. A process for the preparation of triglyceride compositions, in which more than 40 wt.% of the total amount of saturated fatty acids present are in the 2-position, by enzymic interesterification of triglycerides high in trisaturates (= A) with a source providing unsaturated acid moieties (= B), characterized by a multi-step process comprising the steps of :

1) converting triglycerides A enzymatically with a 1,3-specific enzyme and the unsaturated acid source B in a first enzymic conversion zone;

2) removing the spent unsaturated acid source B from the crude product of 1);

3) optionally subjecting the remaining part of 2) to an enzymic removal of diglyceride;

4) converting the remaining part of 2) or the product of 3) in a second enzymic conversion zone with a fresh

source providing unsaturated acid moieties (B) in the presence of a 1,3-specific enzyme;

5) removing the spent unsaturated acid source B from the crude product of 4);

6) optionally recirculating the spent unsaturated acid source (B) from 5) to step 1);

7) decreasing the level of trisaturates ( $S_3$ ,  $S = C_{16}$  or higher) in the remaining part of 5) by a further enzymic treatment, using a 1,3-specific enzyme with an oil blend high in triglycerides with acids other than palmitic and/or stearic acid in the 1,3-positions, but not being a triglyceride composition with more than 40 wt% of the fatty acids in the 2-position being saturated fatty acids with 16 or more C-atoms.

3. A process according to Claim 1 or 2, wherein a fatty acid mixture high in oleic acid is used as a source of the unsaturated acid moieties B.

4. A process according to Claim 2, wherein weight ratios of trisaturated fat A : unsaturated acid source B of 1:2 - 2:1 are used in the first and/or second enzymic conversion zones of steps 1) and/or 4).

5. A process according to Claim 2, wherein in step 3) an Amano G-type enzyme is used.

6. A process according to Claim 1 or 2, wherein a blend is used selected from the group consisting of MCT-oils (medium chain triglyceride;  $C_8$ - $C_{14}$  fatty acids), coconut oil, palm kernel, soybean oil, olive oil, high-oleic sunflower oil, fish oil, rapeseed oil, palm oil and butterfat, or fraction thereof.

7. A process according to Claim 1 or 2, wherein mixture A comprises a mixture rich in palmitic acid with more than 60 wt.% of  $S_3$  (S = palmitic and/or stearic) and more than 20 wt.% of SSU (U = unsaturated acid).

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 5 C11C3/10

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 5 C11C A23D A23C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 417 823 (UNILEVER NV) 20 March 1991 see claims 4,6,12; example ---	1
A	EP,A,0 209 327 (UNILEVER PLC) 21 January 1987 cited in the application see the whole document ---	1
A	EP,A,0 496 456 (UNILEVER NV) 20 July 1992 cited in the application see the whole document ---	1
A	US,A,5 061 498 (NARIHIDE MATSUZAKI ET AL.) 29 October 1991 see claims 1,4,6,17 -----	1

☐ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents :

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